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Exposure Study of an Environmental Mixture formed by Bentazone and Venlafaxine in Mussels



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1. SUMMARY

Mixtures of contaminants, including herbicides and pharmaceutically active compounds (PhACs), are infused into the aquatic environment every day due to anthropogenic activities. These mixtures can be accumulated in non-target organisms highly consumed in the human diet, such as shellfish. Nowadays, information on the bioconcentration capacity of this kind of organisms is limited, although, every day efforts are made to expand the sources of information regarding this matter. In this context, the aim of this work was to provide new information on the bioconcentration and elimination capacity of this type of contaminants mixture in Mediterranean mussels (*M. galloprovincialis*), a commonly consumed shellfish.

For this purpose, an in vivo exposure experiment was undertaken in an Artificial Marine Mesocosm (AMM). Mussel were exposed to a mixture formed by bentazone (BEN), an herbicide; and venlafaxine (VEN), a pharmaceutically active compound used as psychiatric drug. The exposure lasted 15 days followed by 6 days depuration period. Water and mussel samples were taken at different sampling times. The analysis of biota was accomplished by QuECHERS (**Q**uick, **E**asy, **C**heap, **E**ffective, **R**ugged & **S**afe) while water samples were extracted by using Solid Phase Extraction (SPE). The final detection and quantification of the sample were done with Ultra-High-Performance Liquid Chromatography-High Resolution Mass Spectrometry (UHPLC-HRMS).

Concentrations of BEN and VEN in mussel reached up to 1116.16 ng/g dry weight (dw) and 15937.83 ng/g dw, respectively, when expose to an average concentration of 44.98 ng/mL of BEN and 19.42 ng/mL of VEN.

BEN bioconcentration factor obtained was 5.74 L/Kg dw with a 99.81 % of elimination of the compound from the organism in six days. On the other hand, VEN bioconcentration factor resulted in 280.55 L/Kg dw with a percentage of clearance of 98.23 % of the compound in the same depuration period.

The rate constants of uptake (k_1) of BEN and VEN, were 1.65 (L/Kgd) and 1012.84 (L/Kgd), respectively. The rate constant of elimination (k_2) of both compounds were found to be similar 2.23/d and 2.30/d, respectively. The half-life time were also similar 1.55/d for BEN and 1.60/d for VEN.

The results revealed low bioconcentration capacity for both compounds being considered according to their BCFs as non-bioaccumulative compounds. Besides, their depuration rates were high with a 95% of elimination reached in only 48h according to their K_2 values.

Keywords : Bioconcentration, depuration, *M. galloprovincialis*, bentazone, venlafaxine, pharmaceuticals, pesticides, mixtures.

2. RESUM

Les mescles de contaminants perillosos, incloent herbicides i compostos farmacèutics actius (PhAC), s'han introduït cada dia al medi aquàtic a causa de les activitats antropogèniques. Aquestes mescles es poden acumular en organismes molt consumits en la dieta humana, com ara el marisc. Avui dia, la informació sobre la capacitat de bioconcentració d'aquest tipus d'organismes és limitada, tot i que cada dia s'esforcen per ampliar les fonts d'informació sobre aquest tema. En aquest context, l'objectiu d'aquest treball va ser proporcionar informació sobre la capacitat de bioconcentració i eliminació d'aquest tipus de barreja contaminant en els musclos mediterranis (*M. galloprovincialis*), un marisc consumit habitualment.

Amb aquesta finalitat, es va dur a terme un experiment d'exposició in vivo en un Mesocosme Artificial Marí (AMM). Els musclos van ser exposats a una barreja formada per un bentazona (BEN), un herbicida; i venlafaxina (VEN), un compost actiu farmacèutic (PhAC) utilitzat com a medicament psiquiàtric. L'exposició va durar 15 dies, seguida d'un període de depuració de 6 dies. Es van prendre mostres d'aigua i de musclos en diferents temps de mostreig. L'anàlisi de la biota va ser realitzat per QuEACHERS (ràpid, fàcil, barat, eficaç, robust i segur) mentre es van extreure mostres d'aigua mitjançant extracció de fase sòlida (SPE). La detecció i quantificació finals de la mostra es van realitzar amb cromatografia líquida d'alt rendiment alt-Espectrometria de masses d'alta resolució (UHPLC-HRMS).

Les concentracions de BEN i VEN al musclo es van registrar fins a 1116,16 ng / g (pes sec) i 15937.83 ng / g dw, respectivament, quan s'exposen a una concentració mitjana de 44,98 ng / mL de BEN i 19,42 ng / mL de VEN.

El factor de bioconcentració BEN obtingut va ser de 5,74 L / Kg dw amb un 99,81% d'eliminació del compost de l'organisme en sis dies. D'altra banda, el factor de bioconcentració VEN va donar com a resultat 280,55 L / Kg dw, amb un percentatge de depuració del 98,23% del compost en el mateix període de depuració.

Les constants de velocitat d'absorció (k_1) de BEN i VEN, van ser 1,65 (L / Kgd) i 1012,84 (L / Kgd), respectivament. La constant d'eliminació de la velocitat (k_2) d'ambdós compostos es va trobar que era similar a 2,23 / d i 2,30 / d, respectivament. La durada de la vida mitjana també va ser similar a 1,55 / d per a BEN i 1,60 / d per a VEN.

Els resultats van revelar una baixa capacitat de bioconcentració per als dos compostos considerats segons els seus BCF com a compostos no bioacumulables. A més, les seves taxes de depuració eren elevades, amb un 95% d'eliminació en només 48 hores segons els seus valors de k_2 .

Paraules clau: bioconcentració, depuració, *M. galloprovincialis*, bentazona, venlafaxina, productes farmacèutics, pesticides, mescles.

3. INTRODUCTION

Aquatic ecosystems have been suffering changes worldwide associated with human activities (1). Some of these activities require the use of thousands of chemicals, continuously and increasingly infused to these ecosystems by several pathways, creating a mixture of hazardous contaminants. These contaminants -Pesticides and PhACs among them- can also be transfer to shellfish, representing a direct hazard to marine biota, and therefore to humans through the food chain (2), because of their bioaccumulation potential, bioactivity and persistence.

Polar pesticides and PhACs are predominant in European hydro systems (3). They are found in all aquatic compartments at concentrations reaching from few nanograms per liter in coastal marine waters of developed countries (4), up to several dozens of micrograms per liter in surface waters and urban effluents (5) (6).

For some of these chemical substances no maximum levels have been laid down in EU legislation (7). This is the case of herbicide BEN and pharmaceutical drug VEN, the contaminants selected for the present investigation. Keeping in mind this, since toxic effects produced by BEN and VEN in marine biota have been previously observed, it is important to evaluate their bioaccumulation potential in a highly consumed shellfish type such as mussels (*Mytilus galloprovincialis*) in order to assess their environmental and human health risks. Thus, the main aim of the present study was to determine the capacity of bioconcentration and elimination of these compounds in mussels exposed to known concentrations of BEN and VEN.

3.1. Exposure to contaminants mixture in aquatic environments

A large range of contaminants are released into the aquatic environment around the world as a consequence of anthropogenic activities. Herbicides and PhACs are among the different groups of contaminants present in surface, groundwaters, coastal areas and even at open sea. Its transportation into the aquatic environment occurs by different pathways.

Herbicides applied in agriculture, may distribute over the plot of land to which they are applied and introduced into the environment by soil, ground water, surface water, sediment and air (8). Among the transport processes, leaching and runoff are the most notorious processes. Surface water contamination is favor by surface runoff, since the molecule is carried and adsorbed to eroded soil particles or in solution (1). Differently, contamination of groundwater is a result of leaching in which chemical substances, are carried in solution with the water that feeds the ground water (9). Only a small percentage of the herbicides used in soil has a real bioactive effect, the rest of applied product is distributed in the environment (1).

PhACs, on the other hand, enter the aquatic environment through hospital effluents, direct disposal of unused or expired drugs, manufacturing, landfill leachates, livestock activities, aquaculture, soil fertilization with sewage sludge and/or livestock (10). Nevertheless, waste water treatment plants (WWTP) discharges are considered to be the main pathway for PhACs (11). The reason is, these substances are not completely

removed during conventional wastewater treatments (12) and thus, they are discharged to the aquatic environment.

During the transport to open sea, PhACs are subjected to natural attenuation processes. The attenuation is mainly attributed to dilution, while sorption to sediments showed to be a minor pathway, as highlighted by the low number of PhACs detected in these matrices (13). Since their high transformation/ removal rates in the environment are compensated by their continuous introduction into the aquatic systems, PhACs compounds are known as “pseudopersistent” contaminants, leading to a prolonged exposure to aquatic organisms (14).

The different sources and pathways in which contaminants enter the aquatic environment, whether are herbicides compounds, PhACs or others, deliver a mixture of dynamic pollutants that can be affected by seasonal or/and monthly variations, location variations, as well as other factors, such as acidification and/or climate change. The contaminants present in the mixture can have antagonistic and synergistic interactions (15), making them much more toxic to marine biota, than the chemicals alone. The significant combination effects of substances can occur even if the toxicity of the single substances is low.

In regards to this matter, the toxicity assessment done by Di Poi et al. 2017, in marine oyster *Crassostrea gigas*, proved that a mixture of herbicides and PhACs, were much more toxic than exposure to the same chemicals singly (3). Other studies have shown than mixture of the same group, might have stronger toxic effects as well that the compounds separately. A study done by Galus et al. 2012 in adult zebrafish *Danio rerio*, exposed to a mixture of PhACs – venlafaxine among them- showed a significant decreased in embryo production after 6 weeks of exposure (16).

3.2. Compounds of study: bentazone and venlafaxine

The binary mixture selected for the present study consisted of two compounds: bentazone and venlafaxine.

BEN is a commercially marketed and extensively used acidic herbicide for agriculture purposes. It is used for selective control of broadleaf weeds and sedges (17). Its mechanism of action is through the inhibition of photosynthesis (8). It is steady against hydrolysis, however, it can be easily eliminated by photolysis or by biodegradation, by bacteria and fungi. It does not persist in soils; therefore, its high mobility represents a pollution risk for groundwater. In bodies of water exposed to sunlight its half-life is less than 24 hours (18).

VEN, on the other hand, corresponds to the therapeutic family of psychiatric drugs among PhACs. It's widely used for the management of major depressive disorder, generalized anxiety disorder, social anxiety disorder, panic disorder, vasomotor symptoms in women with breast cancer and in postmenopausal women, and neuropathic pain (19).

The compound and its metabolites are eliminated by renal elimination as primary route of excretion. Approximately 87% of a venlafaxine dose is recovered in the urine within

48 hours as either unchanged venlafaxine (5%), unconjugated metabolite O-desmethylvenlafaxine (ODV) (29%), conjugated metabolite ODV (26%), or other minor inactive metabolites (27%) (20).

The chemical structures and physical-chemical properties of BEN and VEN are presented in **Figure 1** and **Table 1** respectively.

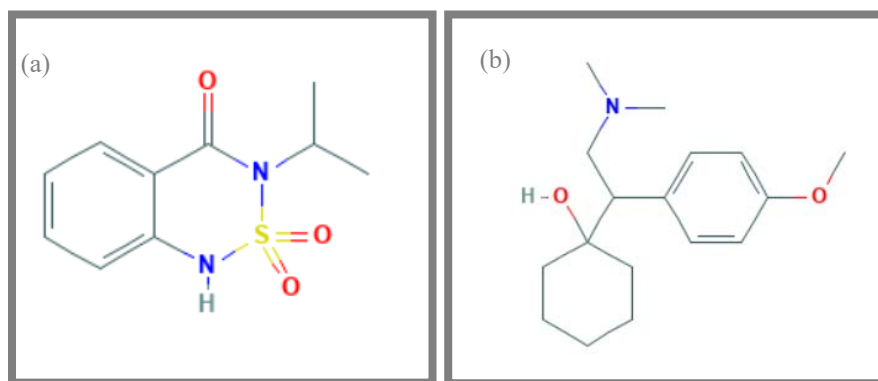


Figure 1. (a) Chemical structure of BEN. (b) Chemical structure of VEN. *Source (a):* <https://pubchem.ncbi.nlm.nih.gov/compound/bentazon#section=Structures>. *Source (b):* <https://pubchem.ncbi.nlm.nih.gov/compound/venlafaxine>

Table 1. Physical-chemical properties of BEN and VEN.

Properties	BEN	VEN
Molecular formula ¹	$C_{10}H_{12}N_2O_3S$	$C_{17}H_{27}NO_2$
Molecular weight ¹	240.277 g/mol	277.408 g/mol
Solubility in water ¹	268.6 at 25°C (mg/L)	266.7 (mg/L) at 25°C
pK_a	3.3. at 24°C ²	10.09 (amine)(est) ⁵
Log K_{ow} ¹	2.34 ³	3.28 ⁶
BCF ¹	16.25 L/Kg ww ⁴	67.85 L/Kg ww ⁴
Experimental BCF	Non reported	213.3-528.1 ⁷

¹ From EPI Suite Software.

² O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Cambridge, UK: Royal Society of Chemistry, 2013., p. 185

³ Experimental by Saito, H et al. (1993) (from EPI suite Software)

⁴ From regression-based method (from EPI Suite Software)

⁵ Reference PubChem

⁶ Estimated (from EPI Suite Software)

⁷ BCF (L/Kg) was obtained by Serra-Compte et al. (2018) experimentally in mussels within different treatments

The compounds which are the subject of this study were selected based on their occurrence and ubiquity in aquatic environments. Their solubility in water as well as other physical-chemical properties related to their bioconcentration potential in marine biota, such as their Log K_{ow} values, are shown in **Table 1**.

Regarding their occurrence in aquatic environments, BEN concentrations vary from non-detected - in open sea water in the Mediterranean Sea and the Black Sea (21)- to 0.15 ng/L in open sea water of the Adriatic Sea (22). Median concentration of 19 ng/L were reported in a study carried out at 20 locations along the coastline of Catalonia. From this study, Delta del Ebro bays had the highest concentrations reported with a detection

frequency of more than 50% of the samples, a mean concentration of 134 ng/L and a maximum concentration of 514 ng/L. This results are in agreement with a previous study, carried out a decade earlier, where pesticide contamination was dominated in the area by herbicides MCPA and bentazone, although the concentrations registered at that time, were double (13).

On the other hand, venlafaxine is frequently detected in the aquatic environment. In a study carried out in different locations in Catalonia, concentrations were found in WWTP influents up to 579 ng/L; in WWTP effluents up to 376 ng/L; in river waters, up to 45 ng/L and in seawater from the Mediterranean Sea, at 52 ng/L (23). In accordance with the results, an investigation studying 81 PhACs, carried out in Tarragona, Spain, showed VEN among the compounds with the highest maximum concentrations detected in the effluent of WWTP, up to 1.2 µg/L. The recovery showed that VEN was one of the compounds with poorest reduction rates (<35%) during the treatment process (24), been discharged into surface waters, reaching coastal areas and therefore, affecting none-target marine biota.

Regarding environmental levels of BEN in marine organism, few studies have been published. The levels found ranged from non-detected in mussels *Mytilus galloprovincialis* and *Anodonta cygnea*; and oyster *Crassostrea virginica* (13) up to 1.56 ng/g dry weight in oyster *Crassostrea gigas*, meanwhile, in cockle *Cerastoderma edule* was found up to 5.11 ng/g dry weight (2). In another field studied recently carried out in Alfacs and Fangar bays, Terrado Rourera et al. 2018 reported BEN was found to be the most ubiquitous compound, present in mussels *Mytilus galloprovincialis* in 7 out of 8 sampling points. Concentrations ranged from 1.07 - 4.20 ng/g dw in Alfacs Bay and 5.52 to 9.36 ng/g dw in Fangar Bay, registering one of the highest concentrations among all compounds.

In the same investigation, VEN was not detected in mussels from Alfacs Bay, however, it was found in mussels from Fangar Bay in 3 out of 3 sampling points at concentrations ranging from 0.78 – 1.29 ng/g dw (25). A higher concentration was reported in the same area when assessing clams and oysters from the Alfacs Bay and mussels from Fangar Bay (26). In this paper, the most ubiquitous compound detected was VEN, with the highest concentrations found in mussel *Mytilus galloprovincialis* up to 2.7 ng/g dw, oyster *Crassostrea gigas* up to 2.3 ng/g dw and clams *Chamelea gallina* up to 2.1 ng/g dw (26). A similar concentration in mussel (2.76 ng/g dw) was reported later on in the same area by Álvarez-Muñoz et al. (2018) (27).

Previous results are consistent with concentrations found by Martínez-Bueno et al (2013), in marine mussels *Mytilus galloprovincialis* collected from the Mediterranean Sea in southeastern France. VEN were occasionally detected at concentrations between 2.5 – 3.7 ng/g dw (28).

In regards to toxic effects in biota, it has been previously observed, that BEN had statistically significant effect on organism *Chironomus riparius* larval weight, and head capsule length after an exposure to sediment during 10 days. Moreover, survival of the larvae decreased toward the highest concentrations (29).

Another study in the algae *Isochrysis galbana* growth rate, and Japanese oyster *Crassostrea gigas* embryo-larval development, tested in seawater either as pure

compound as well as commercial formulation (Opus and Basamaïs) showed that *C. gigas* is sensitive to BEN while both organisms are sensitive to both commercial formulations. Bentazone do not show toxic effect when bioassays were performed at marine environmental concentrations ($<1\mu\text{g/L}$). However, commercial formulations Basamaïs resulted to be 10 times more toxic than bentazone (30).

Venlafaxine, on the other hand, has been reported to affect behavior and reduce survival in fish (31). The compound resulted to be very toxic ($EC_{50} < 1\text{ mg L}^{-1}$) (32) to Pacific oyster *Crassostrea gigas* larvae, a marine organism, and harmful ($10\text{ mg L}^{-1} < EC_{50} < 100\text{ mg L}^{-1}$) (32) to *algae* and *daphnia*, freshwater species (3).

3.3. Target organism: mussels *Mytilus galloprovincialis*

Mussels are bivalves' mollusks (33), sedentary filter feeder animals, feed on small food particles, present in water columns or sediments. They are able to filter different amounts of water depending on several factors, including species (34). *Mytilus galloprovincialis*, for instance, has a filtration rate of up to 2.5 liters of water per hour (35), while *Mytilus edulis* can filter up to 5 liters of water per hour per individual (36).

During this intense filtering activity, bivalve retain plankton necessary for their metabolism, as well as bacteria, viruses and parasites that may be present in the environment (35). These characteristics as sessile and filter-feeding organisms, make them prone to bioaccumulate also contaminants that may be dissolved in the water column or absorbed to suspended organic matter (including herbicides and pharmaceutical drugs). In this way, mussels are valuable for ecological studies (26) (37) and extensively used as sentinels organism for chemical pollution monitoring in natural environments (38). For example, *Mytilus galloprovincialis* was used as the bioindicator specie by The Spanish Institute of Oceanography (IEO) during a monitoring program along the Mediterranean coast of Spain in the 1990s (25). They are also used in the Mussel Watch Program of the United States to provide information for assessing the potential risk to marine wildlife and humans through the use of coastal resources (39).

Marine mussels have also a significant commercial value. Their production corresponds to 50% of global EU aquaculture in weight and about 30% in value, been, the Mediterranean (*Mytilus galloprovincialis*) and the blue (*Mytilus edulis*) mussels the most important species (40) due to their significant consumption by the population.

Thus, this sedentary organism, with a high filtration capacity, considered as biomonitoring species and of high relevance for human's diet, was selected as target bivalve species for the present study.

3.4. Bioconcentration, uptake and depuration

Chemicals can be accumulated in organisms through different routes. The general term that describes a process, by which chemicals are taken by an organism, either directly by

exposure to a contaminated medium, or by the consumption of foods containing the substance chemistry, is called bioaccumulation (41) .

A special case of non-dietary bioaccumulation of a substance dissolved in water (42), which results in a greater concentration in the body than in water (43), is referred to as bioconcentration and is measured by the Bioconcentration factor (BCF).

BCF is the most used parameter to assess contaminants uptake by organisms which relates the concentration of a certain contaminant in biota with its corresponding concentration in water (44), and is calculated through the following formula as well as the Bioaccumulation factor (BAF), when given in an aquatic environment (45):

$$BAF, BCF = \frac{C_{organism}}{C_{water}} ,$$

Where, $C_{organism}$ is the concentration of the compound in the organism, while C_{water} is the concentration of the compound in water. A stationary state is reached when the rate of transfer of matter towards the interior and exterior of the organism is equal and, therefore, no net change in its concentration occurs (43).

Regulatory authorities, like the Environmental Protection Agency (EPA) and the European (EU) Commission Regulation, established a BCF higher than 1000 L/Kg wet weight (ww) (46), or than 2000 L/Kg ww (47) as threshold for considering a compound to be bio-accumulative in organisms. Therefore, when a BCF is below 1000 or 2000 L/Kg, the compound is considered as non-bioaccumulable, between 2000 and 5000 is considered as bioaccumulable and above 5000 as very bioaccumulable (43).

In accordance to these criteria Stockholm Convention established the BCF in aquatic species greater than 5000 or in the absence of data, with a $\log K_{ow}$ greater than 5, results in highly bioaccumulative compounds (45).

Initially, bioaccumulation potential was estimated only, by the n-octanol-water partition coefficient in its logarithmic form ($\log P_{ow}$ referred in Medicinal Chemistry or $\log K_{ow}$ referred in environmental sciences), that simulates the hydrophobic character or lipid affinity of the compound. Nowadays, it continues to be a very used parameter. Its value is related to its adsorption capacity or bioconcentration potential in fatty tissues. Thus, a value of K_{ow} greater than 5 describes a high affinity of the compound for fatty tissues in animals, therefore, bioconcentration or bioaccumulation is likely. Its low mobility favors the toxicity of the compound. A value between 3 and 5, describes a medium affinity and a value less than 3, describes a low affinity indicating the probable mobility and transport of that material due to its good solubility, and easy metabolism and biodegradation, therefore, a low bioaccumulation would be expected.

Thus, bioaccumulation results from complex interactions between various routes of uptake, excretion, passive release, and metabolism. For aquatic organisms, the bioaccumulation process includes the previously mentioned routes of uptake: aqueous uptake of water-borne chemicals, and dietary uptake by ingestion of contaminated food particles. Bioconcentration, on the other hand, can be viewed simply as the result of the competing rates of chemical uptake and elimination.

The uptake of contaminants is most often driven by passive diffusion processes from the water body through gills, into living tissues. The excretion processes include passive diffusion across biological membranes and active or facilitated transport in organs of elimination – such as hepatobiliary system, gill, and kidney-. Factors determining gill excretion include proper lipid solubility (e.g., rapid excretion of chemicals with $\log K_{ow}$ between 1 and 3) (48). In **Figure 2** a conceptual model of bioconcentration from water in an aquatic organism is presented.

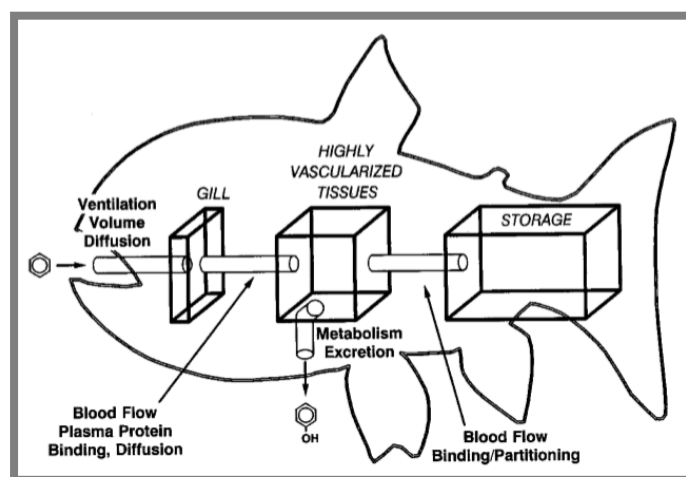


Figure 2. Conceptual model of bioconcentration from water. *Source (a): Barron, M.G., Environ. Sci. Technol., 24, 1612, 1990.*

To calculate the uptake and elimination of a contaminant, rate constants are used, called K_1 and K_2 , respectively. The uptake and elimination rate constants, relates the concentration of the contaminant in water and the concentration of the contaminant in the whole body of the organism in a specific time.

In addition to the BCF and the octanol-water partition coefficient, the bioaccumulation potential also depends on the depuration expressed as half-life clearance time (CT_{50}). This is, time needed to reach 50% removal of the compound (49), and factors such as hydrophobicity of the compound, species, size and age of the organisms, presence of dissolved organic matter, chemical-physical properties of the environment and structure of the compound (43).

The percentage of depuration in an organism from a specific compound varies from one compound to another as one can be more bioaccumulated than others. As some contaminants can be dangerous to human health, especially when shellfish are eaten raw or under-cooked, EU Regulations, in an attempt to limit the risk in the human food chain, require that a purification treatment be performed prior to the trade of bivalve mollusks. Such a process consists in a short relaying period of the mollusks in tanks, where they can filter clean sea water for 24 hours. This process can be affected by several factors (35) making one day not enough time for reaching a complete depuration from contaminants as it has been observed by Serra-Compte et al. 2018 (50) for sulfamethoxazole in mussels.

4. OBJECTIVES

The main goal of this research was to evaluate and characterize the bioconcentration and depuration, of a mixture of bentazone and venlafaxine (contaminants of emerging concern) in mussels (*Mytilus galloprovincialis*), exposed to known concentrations of these contaminants. For this purpose, an in vivo exposure experiment was carried out during twenty-one days under laboratory conditions in which.

The specific objectives of the research were:

- To determine the bioconcentration factors of BEN and VEN in mussels.
- To calculate the experimental kinetics of incorporation and elimination (K_1 and K_2) of both contaminants in mussels.
- To measure the percentage of depuration of both contaminants in mussels after 6 days of clearance.
- To determine the half-life time of BEN and VEN in mussels.

5. MATERIAL & METHODS

5.1. Experimental approach

5.1.1. Reagents and organism

The standards used BEN and VEN were purchased from Sigma Aldrich and were of high purity grade (>90%). For the internal standards, isotopically labelled compounds were used and purchased also from Sigma Aldrich. Both, individual stock standards and isotopically labelled standards were prepared in methanol at a concentration of 10 µg/mL. Preparation in 100% acetonitrile (ACN) for working standards solutions of 1 µg/mL, containing either standards or isotopically labelled internal standards were done before each analytical run.

Bekolut (Barcelona, Spain) kindly supplied the QuEChERS Bekolut Citrat-Kit-01 and Bekolut PSA-Kit-04A. HPLC grade water and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany) and the Ostro™ 96 well plate from Waters (Barcelona, Spain).

All Mediterranean mussels, *Mytilus galloprovincialis* were selected based on the minimum size for commercialization, consequently, all individuals used in the study exceed four centimeters in size. Organisms were collected from an aquaculture facility located at approximately three kilometers away from the coast of the Alfacs Bay (south of Ebro Delta, Catalonia, Spain) in March 2019, and were immediately transported in refrigerated conditions to the Institute of Environmental Assessment and Water Research IDAEA-CSIC (Barcelona, Spain), where the experiment was carried out.

5.1.2. Artificial Marine Mesocosm experiment (AMM)

The AMM is composed by small experimental units that replicate aspects of the natural environment as closely as possible (51). In this particular study, it was set up to reproduce the Mediterranean coastal environment in a closed system. It is formed by 4 units of 500 liters each, accounting for a total volume of sea water of 2000 liters for the entire AMM (units shown in **Figure 3A**).

Each unit is formed by the main tank, cooling system, skimmer, biological filter and pumps to move water between the different compartments (**Figure 3A, B and C**). The main tank is where the organisms were located. The cooling system kept the water at the desired temperature (17 ± 0.1 °C), maximum temperature registered in the Mediterranean Sea (Barcelona Coast), during April 2019 (52). The skimmer, located in a second tank, recreated nature's own effect by adhering and raising particles of proteins, trace elements, and other organic waste to the surface by introducing air bubbles through a flow of water. In this second compartment, the biological filtration took place. The compartment was composed of active stones with nitrifying bacteria, which transform the ammonium to nitrite, by oxidation of the Nitrosomonas and from nitrite to nitrate, by the oxidation

generated by *Nitrobacter*. Pumps and gravity were used to move the water around the different parts that composed the unit.

All compartments were interconnected between them. From the main tank, water was carried out to the cooling system by a pump and then, returned to the main tank at the desired temperature. When water exceeded the limit level of the main tank, it descended by a pipe to the lower tank (by gravity). In this second tank there was a first compartment where the filtration of sea water occurred through the skimmer. Then, the water passed to a second compartment where the active stones were placed and the biological filtration occurred. From this compartment the sea water already filtered was pumped back into the main tank where the organisms were located. Details of the AMM and its different compartments are presented in **Figure 3A, B and C**.

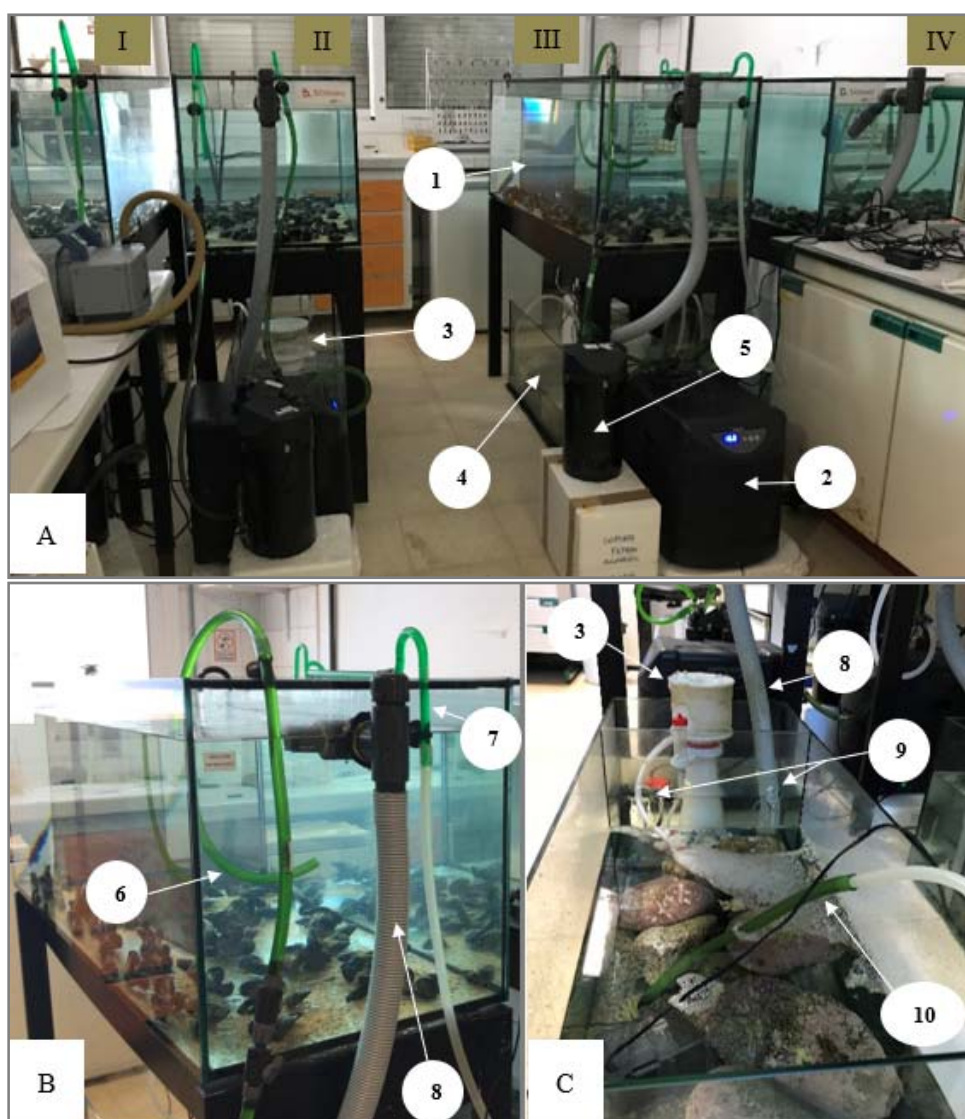


Figure 3. Overview of the Artificial Marine Mesocosm. *Source: Original photos taken by author.*

(A) Units that form the AMM (I, II, III, IV). Each unit is composed by the main tank (1), cooling system (2), skimmer (3), biological filter (4) and pumps (5).

(B) Detail of the inlet and outlet pipes of water in the main tank. Water flow from the main tank to the pump and cooling system (6), water flow from the cooling system into the main tank (7), water flow from the main tank to the skimmer and biological filter (8).

(C) Detail of the biological filter. Skimmer (3), water flow from the main tank to the skimmer (8), water flow from the skimmer to the active stones (9) and water flow from the biological filter (after depuration) to the main tank (10).

Every compartment of the four units of the AMM were filled with seawater, obtained from Platja Sant Pol located in Sant Feliu de Guíxols (Girona, Spain), during the month of March 2019. Two of the units were used as control groups (where no contaminant compound was added) and two of the units were used to expose mussels to the mixture of BEN and VEN.

The system was turned off once a day during the experiment to introduced pollutants by spiking the seawater in the exposed units during the exposure phase, and to feed the organisms in both exposed and control units during the entire experiment.

The mixture of BEN and VEN was added at a final concentration of 10 µg/L for each pollutant via water in the exposure tanks. The first day of the exposure phase, 100% of the initial concentration of BEN and VEN was added. The second day and until the end of the exposure phase, 100% of initial concentration of BEN and 25% of the initial concentration of VEN was added to the exposure tanks, in order to maintain it constant, and compensate the loss due to degradation and/or adsorption of the mixture in the system. These percentages were chosen due to values reported in previous studies, which showed BEN half-live in water was 24 hours when receiving sunlight (18). VEN, on the other hand, was degraded in 25% during 24 hours (44).

Mussels were fed with the recommended dose of 1% of mussel wet weight, with a commercial microalga mix suitable for bivalve mollusks, composed by five species: *Isochrysis spp.*, *Tetraselmis spp.*, *Pavlova spp.*, *Nannochloropsis, spp.* And *Spirulina spp.* (Acuinuga, Spain).

The water loss in the system due to evaporation during the filtration processes (2% of the volume, approximately) was added to the system when its level was reduced.

Water quality control was done by measuring the temperature, pH, dissolved oxygen, nitrates and nitrites of the units. The results were constant through the experiment. Temperature was 17 ± 0.1 °C, pH 7.5 ± 0.1 units. Dissolved oxygen was over 60% of saturation due to the constant moving of the bodies of water. Nitrates (NO_3^-) were register to be below 25 mg/L and nitrites (NO_2^-) were below 1 mg/L for the exposure tanks. For control tanks, nitrates (NO_3^-) levels were higher at the end of depuration period, with values up to 300 mg/L and also nitrites (NO_2^-) up to 1 mg/L. In this case, a replacement of 10% of the total volume of seawater of the unit was done. Regarding day-night cycle, the organisms were exposed to natural light during spring time, approximately 12h day and 12h night.

The experiment lasted twenty-six days, starting from March 20th until April 15th of 2019, and it was divided in three different periods. First, an acclimation period that lasted 5 days where mussels were not exposed to any contaminant compound, but they were kept in laboratory experimental conditions. Second, for the exposure period, two AMM units

were exposed to a mixture of BEN and VEN for 15 days (360 hours), while other two were kept in control conditions (contaminant mixture free). Third, a depuration period (all units free of contaminants) that was carried out during 6 days (144 h more). In total the duration of the experiment considering exposure and depuration periods was 21 days (504 h).

During the acclimation period, 500 mussels were divided into the four units. Therefore, 125 mussels were placed in each unit for the exposure period. At the end of this stage, the number of mussels remaining in the units for the depuration period was 70. Low mortality, below 1% was observed only during acclimation period. No mortalities were registered during the rest of the experiment.

Regarding the sampling, for BEN and VEN bioaccumulation study, 4 individual's whole tissue and seawater were sampled at 0, 3, 5, 7, 9, 24 (1d), 48 (2d), 120 (5d), 240 (10d), 360 hours (15 d) of the exposure phase, and at 504 hours (21d), end of the depuration phase, from both control and exposure tanks for their chemical analysis.

5.2. Chemical analysis

Mussels were analyzed according to the method developed by Alvarez-Muñoz et al. (2019) (2). Four individuals were taken from each AMM unit at each sampling time. They were combined in two replicate samples formed by 2 mussels each. For the control tanks the analysis was carried out at 0, 15 and 21 days of the experiment. For the exposure tanks the analysis was carried out at 0, 3, 5, 7, 9, 24 (1d), 48 (2d), 120 (5d), 240 (10d), 360 hours (15 d) of the exposure phase, and at 504 hours (21d) at final depuration phase day.

Once the samples were taken, they were weighted and stored at -20 degrees. Later on, they were lyophilized, re-weighted and grounded in a mortar. For their analysis with QuEChERS, 1 g dw per sample was weighted and extracted. Prior to the extraction, 50 µL of the internal standards mixture containing venlafaxine-d6 and bentazon-d7 were added, mixed properly by vortex and left to equilibrate overnight (12h) in refrigerated conditions.

5.2.1. Extraction and purification of samples

The day after the internal standards mixture was added to the samples, QuEChERS method was carried out according to Álvarez-Muñoz et al., 2019 (2). This method consisted on solid phase dispersive extraction system (dSPE) that involves two fundamental stages, a first stage of simple extraction (LLE) followed by a phase of cleaning the extract by dispersion (dSPE) (53).

For the LLE, 10 mL of acetonitrile (ACN) was added to the sample followed by 5 mL of HPLC water together with a mixture of salts containing 4g of MgSO₄, 1g of NaCl, 1g of NaCitrate and 0.5g of disodium citrate sesquihydrate (Bekolut citrat-kit-01). Next, the sample was vortexed for 1 minute at 2500 rpm, and then centrifuged (5 min at 4000 rpm

at 15°C). Once these steps were concluded, 6 mL of the supernatant liquid was transferred to a centrifuge tube of 15 mL to perform the dispersive solid phase extraction (dSPE) by adding QuEChERS Bekolut PSA-Kit-04A.

This second phase consisted of adding 4 mg of primary secondary amine (PSA), 400mg of octadecylsilane (C18e) and 1200mg of MgSO₄. The mixture was vortexed and centrifuged again for 1 min at 2500 rpm and 5 min at 4000 rpm at 15°C, respectively. The supernatant liquid was transferred to a tube to evaporate under nitrogen until complete dryness, then it was re-dissolved in 1 mL of ACN, and filtered through a phospholipid's removal plate for purification named Ostro™. The filtered liquid was transferred to appropriate vials for their injection in (UHPLC-HRMS).

For the water analysis, 10 mL of seawater were taken from each AMM unit at each sampling time. Prior to the extraction, 300 µL of ethylenediaminetetraacetic acid (Na₂EDTA) were added and mixed properly. Immediately after, solid phase extraction (SPE) on Oasis HLB (200 mg, 6 mL) was carried out. The cartridges were conditioned with 6 mL of methanol (*M_eOH*), followed by 6 mL of HPLC grade water. The entire 10 mL of sample were loaded. The cartridges were rinsed with 6 mL of HPLC water, dried with air for five minutes, eluted with 6 mL of *M_eOH* and stored at -20 °C until analysis. Prior to the analysis, the samples were dried under nitrogen, brought up in 1 mL of ACN and 50 µL of the internal standards mixture were added. The filtered liquid was transferred to appropriate vials for their injection in UHPLC-HRMS.

5.2.2. Analysis by Ultra High-Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (UHPLC-HRMS)

The mussel extracts and water samples were analysed by ultra-high-performance liquid chromatography coupled to orbitrap Q-exactive™ high resolution mass spectrometry. Chromatographic separations were carried out with an Acquity Ultra-Performance™ Water liquid chromatograph system from Waters (Milford, MA, USA), equipped with two binary pumps system using for both positive and negative electrospray ionization. Purospher STAR RP-18 end-capped column (150 mm x 2.1 mm, 2 µm particle size) (Merk, Darmstadt, Germany) was used. The optimized separation conditions were a regular flow rate of 0.2 mL/min for BEN and 0.3 mL/min for VEN. Elution gradient of the analytical method developed by Álvarez-Muñoz was slightly modified in order to make the run faster. The gradients are presented in (Table 2 for BEN and Table 3 for VEN. Volume of injection was 20 µL; the column temperature was set at 25°C.

Table 2. BEN Elution gradient for mussel sample. A= ACN B= H ₂ O		
%A	%B	Time (min)
10	90	0
50	50	2.5
80	20	12.5
100	-	13
100	-	14
10	90	14
10	90	16

Table 3. VEN Elution gradient for water sample. A= ACN B= H ₂ O		
%A	%B	Time (min)
10	90	0
50	50	2.5
80	20	7.5
100	-	8
100	-	10
10	90	12
10	90	15

The UHPLC instrument was coupled to a Q-exactive orbitrap mass spectrometer (Q-Exactive™ Thermofischer Scientific, San Jose, CA, USA) equipped with an electrospray ionization source which performed the detection. Full scan data in both positive and negative mode were acquired at a resolving power of 70,000 FWHM.

The peaks of the target compounds in the samples were confirmed by comparing their retention times with those in the standard solutions and also by identifying the precursor ion with a mass error below 5 ppm. Blank samples (100% ACN) were run every 3 samples on the sample queue in order to detect any possible carryover effect.

The concentrations measured in the samples were determined by using internal calibration. For this purpose, a calibration curve ranged between 1 and 50 ng/mL of the target compounds was prepared containing as well 50 ng/mL of the internal standards used (venlafaxine-d6 and bentazon-d7). The quantification was done by using Thermo Xcalibur Software v. 3.1.

5.3. Bioconcentration factor (BCF), incorporation and elimination

The BCF values in L/Kg were calculated based on the following formula described previously:

$$BCF = \frac{C_{biota}}{C_{water}} \text{ L/Kg dw}$$

where C_{biota} is the analyte concentration of BEN and VEN in mussels (μg/Kg dw), whereas C_{water} is the analyte concentration of BEN and VEN in water (μg/L). The BCF were calculated for each sampling time during the experiment. The percentage of the mixture elimination during the depuration phase was estimated according to the following equation:

$$\text{Percentage of elimination (\%)} = 100 - \left[\left(\frac{C_{end}}{C_{initial}} \right) * 100 \right],$$

where C_{end} is the analyte concentration of BEN and VEN in mussels (μg/Kg dw) at the end of depuration phase (day 21), whereas $C_{initial}$ is the analyte concentration of BEN and VEN in mussels (μg/Kg dw) at the end of exposure phase (day 15).

To calculate the incorporation and elimination kinetics, a model of a first-order reversible kinetic was used to determine the uptake of the compound in the exposure phase:

$$\frac{-dC_w}{dt} = \frac{dC_o}{dt} = k_1 C_w - k'_2 C_o \quad (1)$$

where C_w is the concentration of contaminant in water ($\mu\text{g/L}$); C_o is the concentration of contaminant in the whole-body organism ($\mu\text{g/Kg}$); k_1 is the uptake rate constant (L/Kgd); k'_2 is the elimination rate constant during the exposure phase ($1/\text{d}$), and t is the time (h).

When the steady state and hence the equilibrium was achieved:

$(C_o)_t = (C_o)_e$ and $\frac{d(C_o)_t}{dt} = 0$, giving:

$$\ln \frac{(C_o)_e}{(C_o)_e - (C_o)_t} = \frac{k_1(C_w)_o}{(C_o)_e} t \quad (2)$$

The utilization of this formula, enabled k_1 calculation by using the experimentally determined values of contaminant concentration in the water and in the organism.

The rate of loss of the contaminant compound k_2 , was calculated by using a first-order decay equation during the depuration phase, when there was not incorporation of the contaminant and the elimination took place:

$$\frac{-dC_o}{dt} = k_2 C_o \quad (3)$$

The kinetic BCF is the quotient between the two rate constants (k_1 / k_2) and was calculated based on this relation. The calculation of the half-life time ($t_{1/2}$) of BEN and VEN were estimated using the formula:

$$t_{1/2} = \left(\frac{1}{k_2}\right)(\ln 2)$$

6. RESULTS AND DISCUSSION

6.1. Contaminants concentrations in experimental seawater and mussel

BEN and VEN concentrations measured in water during the exposure and depuration phases in the AMM are shown in **Figure 4**. Both compounds showed similar behaviour in the replicates mesocosm units used for the exposition, which indicates the good performance of the experimental system (AMM) and the high reproducibility of the

experimental conditions. However, it was observed that although during the first 24 hours of the exposure the concentration of both contaminants in water was the desired one (around 10 ng/mL), after this time the concentrations increased along the experiment. This was due to the spiking strategy used in which 25% of the initial concentration of VEN and 100% of the initial concentration of BEN was added daily. Therefore, the concentration of BEN along the exposure period ranged between 4.51 and 151.02 ng/mL with an average concentration in both mesocosms units (Exposure A and Exposure B) during the exposure phase of 44.98 ng/mL. The levels of VEN in the exposure water ranged from 5.29 to 44.61 ng/mL with an average concentration for both mesocosms units of 19.42 ng/mL. During the depuration phase, BEN and VEN levels were detected at low concentrations with an average value of 0.57 and 1.01 ng/mL respectively for BEN and VEN. In the control mesocosm units, VEN was non-detected along the experiment and BEN was detected in water at concentrations that did not exceed 0.20 ng/mL.

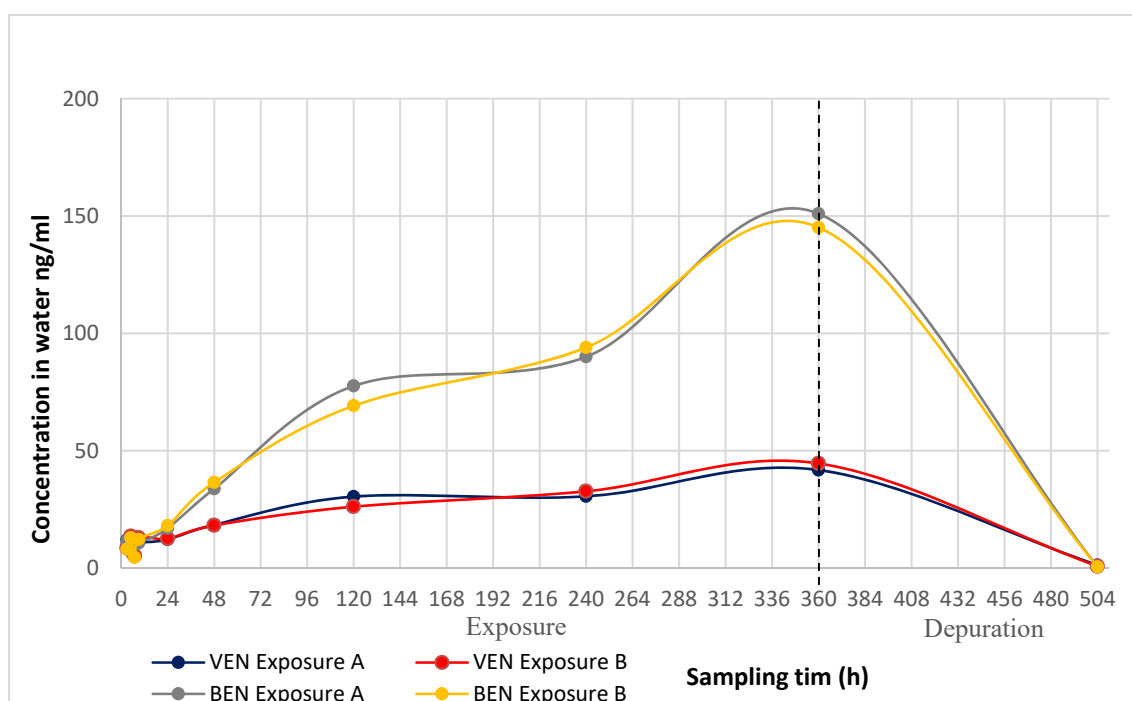


Figure 4. BEN and VEN mean concentrations (ng/mL) in water of exposure tanks along the exposure and depuration periods. Experimental replicates of exposure were performed in two different units of mesocosm called exposure A and exposure B.

The exposure concentrations of BEN and VEN during the experiment were higher than the ones commonly found in the marine environment for these compounds that normally ranged from non-detected to several hundreds of ng/L (as reported in section 3.2) (22) (54) (23). However, ng/mL levels maybe be easily achieved in coastal areas, especially onshore, where mussels are cultivated, and sometimes the shellfish farms are located nearby contamination sources like in the case of Ebro Delta.

Regarding the concentration of BEN and VEN found in water at the end of depuration it was due to the excretion of contaminants by the organisms, as well as the concentration of BEN found in control mesocosms units.

BEN mean concentrations and standard deviation ($n=4$, experimental replicates considering exposure A and B) in mussels during the exposure and depuration period are presented in Figure 5. BEN was present in mussels at the beginning of the experiment (sampling time 0) as well as in the control mesocosms, at an average concentration of 2.97 ± 1.32 ng/g dw. The concentration increased during the exposure phase up to 1116.16 ± 169.50 ng/g dw, after 15 days of exposure (360 hours). During the depuration phase, mussel's concentration decreased to 2.12 ± 1.13 ng/g dw, after 6 days (144 hours) in a free contaminant environment, which resulted in 99.81 ± 0.10 % of elimination when comparing the concentration at the end of the exposure phase with the concentration at the end of the depuration phase.

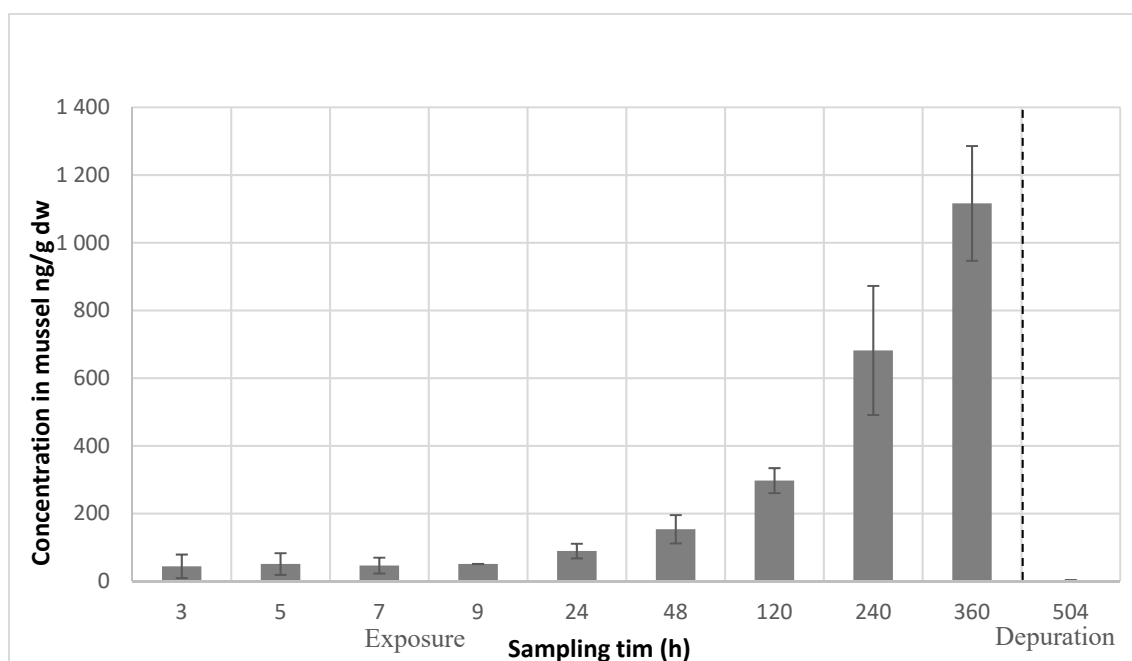


Figure 5. BEN average concentration (ng/g dw) and standard deviation ($n=4$) in mussels from exposure A and exposure B mesocosm units.

The background concentrations found in mussels from the control tanks and at the beginning of the experiment (before the spiking was done) were taken into consideration for calculations, therefore, the results presented in figure 5 are properly corrected. This background levels of BEN found in experimental mussels are in the same range (low ng/g) than the ones previously reported by other authors in Ebro Delta (25) (2) from where the organisms were collected for carrying out the experiment.

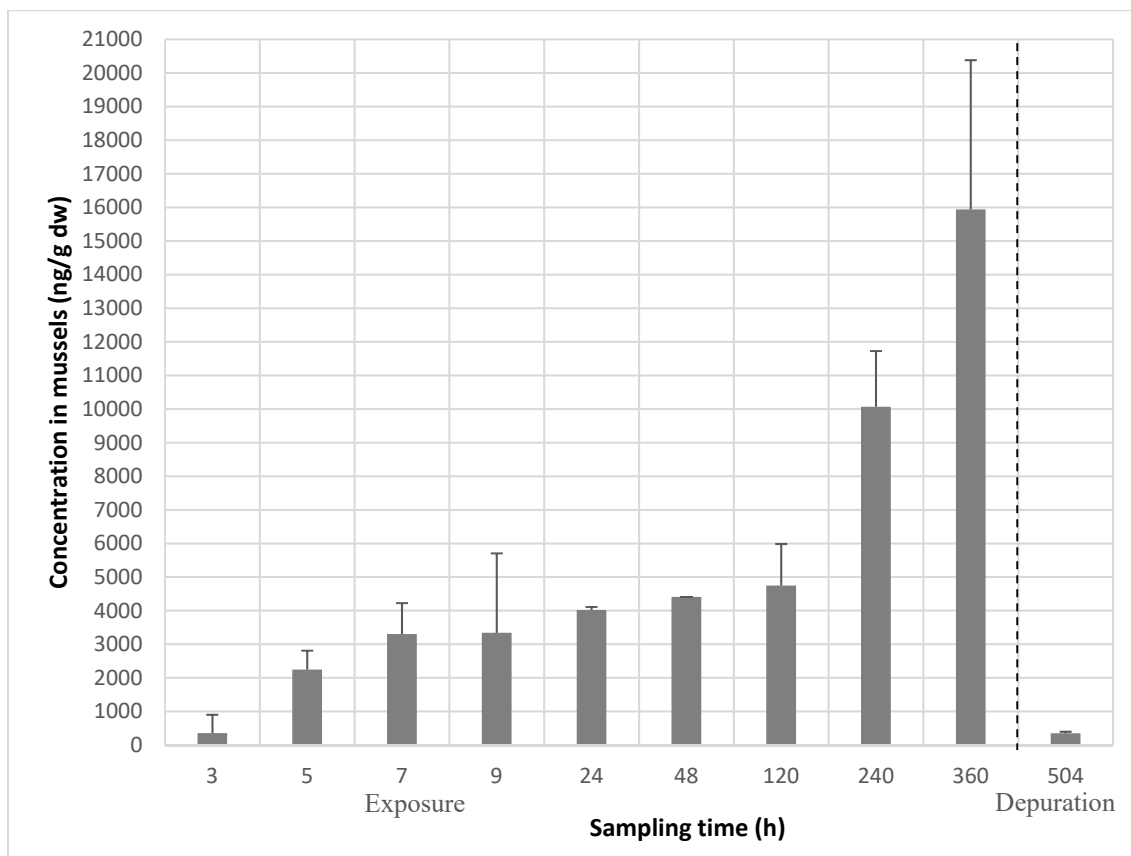


Figure 6. VEN average concentrations (ng/g) and standard deviation (n=4) in mussels from exposure A and exposure B mesocosm units.

VEN mean concentrations and standard deviation (n=4, experimental replicates considering exposure A and B) in mussels during the exposure and depuration period are presented in **Figure 6**. VEN concentration was not present in mussels at the beginning of the experiment, nor was present in the control mesocosms. The concentration of VEN increased during the exposure phase from 354.63 ± 545.96 to 15937.83 ± 4442.84 ng/g dw, after 15 days exposure (360 hours). During the depuration phase, mussel's concentration decreased to 347.17 ± 49.14 ng/g dw, after 6 days (144 hours) in a free contaminant environment, which resulted in 98.23 ± 0.85 % of elimination when comparing the concentration at the end of the exposure phase with the concentration at the end of the depuration phase.

The concentration of VEN found in mussels is in the same order of magnitude than the values previously reported by Serra-Compte et al. (44), where 5419.5 mg/Kg dw of VEN were measured in mussels after 20 days of exposure under laboratory conditions, at an exposure concentration of 10.7 ± 1.6 mg/L.

If the concentration measured in organism of BEN and VEN are compared it can be observed that; a much higher concentration of VEN was bioconcentrated by mussel even when the exposure levels in water for VEN were lower. This is due to the higher LogK_{ow} of VEN compared to BEN that makes this compound more prone to be accumulated in mussel's tissue.

6.2. Bioconcentration factors (BCFs)

Figure 7 shows the BCF variation with respect to the time of exposure for BEN. Steady state was assumed to be reached when the increase of the concentration in the organism divided by the concentration in water (C_o/C_w) was not significant, concretely between 24 h and 48 h after starting the experiment. Therefore, the exposure phase (360 h) was long enough to guarantee the establishment of a steady state, and the calculation of the steady-state bioconcentration factor (BCF_{ss}) (C_o/C_w after the steady-state is reached). Similar results were obtained for VEN (**Figure 8**) where the steady stated was also achieved between 24 and 48 h.

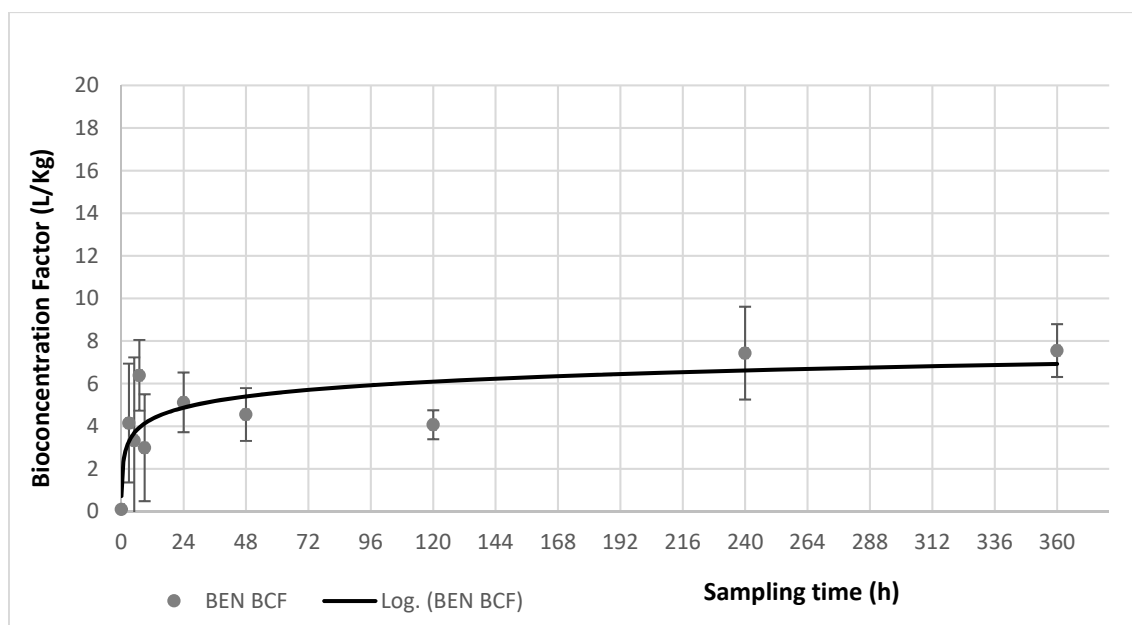


Figure 7. BEN mean bioconcentration factor (BCF expressed in L/Kg dw) in mussels *M.galloprovincialis* and standard deviation (n=4).

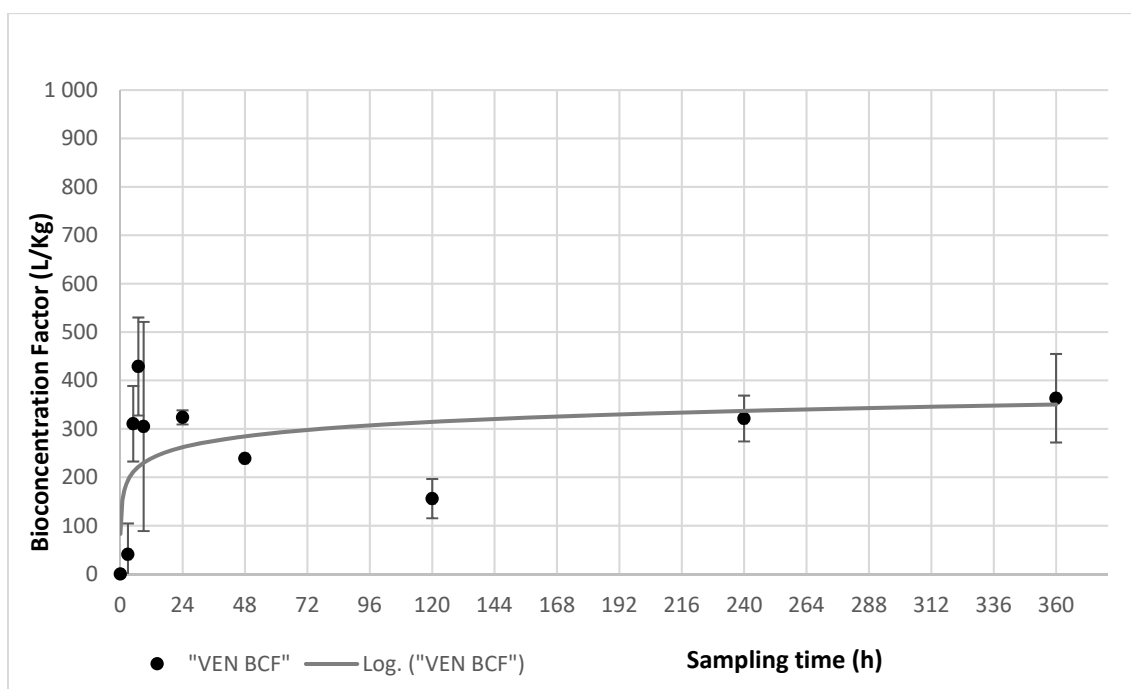


Figure 8. VEN bioconcentration factor (BCF expressed in L/Kg dw) in mussels *M.galloprovincialis* and standard deviation (n=4).

Table 4 presents the BCFs calculated in the steady state and their standard deviation obtained for each compound of the mixture.

Table 4. Bioconcentration factors calculated in the steady state (BCF_{ss} expressed in L/Kg dw), uptake (k_1 , L/kgd) and elimination (k_2 , 1/d) rate constants, and half life time ($t_{1/2}$, d) for *M.galloprovincialis* exposed to BEN and VEN.

Compound	Exposure concentration (ng/g dw)	Time (h)	BCF (L/Kg dw)	BCF _{ss} (L/Kg dw)	k_1 ($\frac{L}{Kg d}$)	k_2 ($\frac{1}{d}$)	$t_{1/2}$ (d)
Bentazone	44.98	3	4.15±2.79	5.74±1.64	1.65	2.23	1.55
		5	7.03±5.32				
		7	6.39±1.66				
		9	2.99±2.51				
		24	5.12±1.40				
		48	4.55±1.24				
		120	4.07±0.68				
		240	7.43±2.18				
		360	7.55±1.24				
Venlafaxine	19.42	3	40.80±63.64	280.55±8 3.17	1012.84	2.30	1.60
		5	310.49±78.10				
		7	428.74±101.26				
		9	304.93±216.12				
		24	323.66±14.67				
		48	238.77±0.00				
		120	155.76±40.58				
		240	321.37±47.40				
		360	363.18±91.46				

For BEN, the BCF values ranged between 2.99 ± 2.51 and 7.55 ± 1.24 L/Kg along the exposure phase. The average value for the BCF obtained during the whole experiment was 5.59 ± 2.06 L/Kg, very similar to the BCF_{ss} , estimated in the steady-state after 24 hours (5.74 ± 1.64 L/Kg). This value is lower than the theoretical BCF estimated with regression-based method presented in EPI Suite Software (16.25 L/Kg) (55), but on the same order of magnitude. It is worth mentioning that the value presented in EPI Suite Software does not specify the species in which it was estimated, nor the author, so it can be an unreliable source of comparison.

For VEN, the BCF values ranged between 40.80 ± 63.64 and 428.74 ± 101.26 L/Kg. The BCFs obtained had an average value of 273.50 ± 105.91 L/Kg considering the whole experiment. A very similar BCF_{ss} was obtained after the steady-state was reached at 24 hours (280.55 ± 83.17 L/Kg). These values were on the same range as the bioconcentration factor presented previously by other author in *M. galloprovincialis*, 213.3-528.1 L/Kg (44); and higher from the theoretical BCF presented in EPI Suite Software (67.8 L/Kg) (56). Once again, EPI Suite Software did not show in which species the BCF was estimated, nor the author.

Comparatively the BCF_{ss} determined for BEN in mussel (5.74 L/Kg) is considerably lower than the one obtained for VEN in the same organism (280.55 L/Kg). This is mainly related to differences in their physical-chemical properties. While BEN has a $\log K_{ow}$ of 2.34 VEN presents a $\log K_{ow}$ of 3.28. As explained previously, a value less than 3, describes a low affinity for fatty tissues indicating the probable mobility and transport of that material due to its good solubility, and easy metabolization and biodegradation, therefore, a low bioaccumulation was expected. Meanwhile, a value between 3 and 5, describes a medium affinity, explaining why VEN was more bioconcentrated. Anyhow, in both cases the BCFs are below 1000 L/Kg and, therefore, they are considered as non-bioaccumulative compounds (43).

6.3. Uptake and elimination

The rate constants of uptake (k_1) and elimination (k_2) of BEN and VEN, were calculated according to the equations described in section 5.3 and they are presented in **Table 4**. BEN showed a value for k_1 of $1.65 \left(\frac{L}{Kg \cdot d} \right)$, a linear regression of the ratio $C_o/C_w(t)$ mean value against time from the initial linear part of the uptake curve was performed, and the correlation coefficient was $R^2=0.9618$. For k_2 , a value of 2.23/d was calculated through a non-linear curve during clearance phase with $R^2=0.9972$, as shown in **Figure 9**. The kinetic BCF was calculated as the quotient between the two rate constants (k_1/k_2), resulting in 0.74 L/Kg, which is smaller but on the same order of magnitude than the BCF for BEN calculated experimentally in the steady state (5.74 ± 1.64 L/Kg).

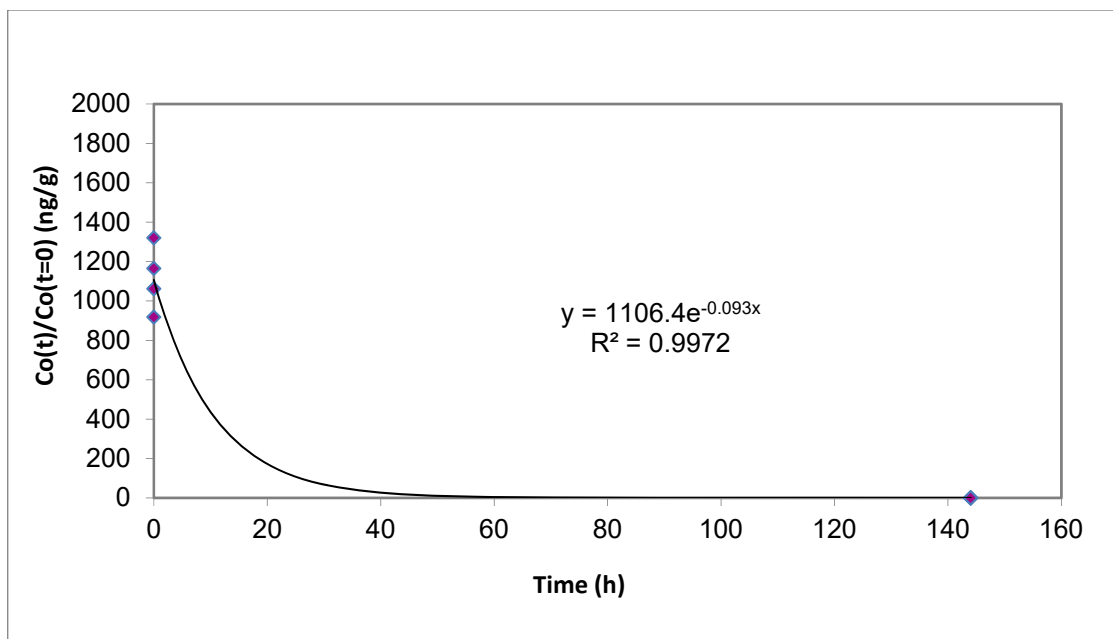


Figure 9. BEN rate constant of elimination calculated in mussels *M.galloprovincialis*.

For VEN, k_1 was estimated in 1012.84 (L/Kgd) with a correlation coefficient of $R^2 = 0.9269$. The values calculated for k_2 was 2.30/d, as shown in **Table 4** with $R^2 = 0.9952$ as shown in **Figure 10**. For VEN the kinetic BCF was determined in 439.22 L/Kg higher than the experimental BCF (280.55 ± 83.17). To the best of our knowledge, this is the first time that k_1 and k_2 have been reported for BEN and VEN in mussels. Since no previous studies have been found, comparison with other values for the same compounds cannot be done. However, if the values are compared among them, it can be observed that VEN has a highest velocity of uptake (k_1), probably related to its higher LogKow, while the value of k_2 is similar for both contaminants.

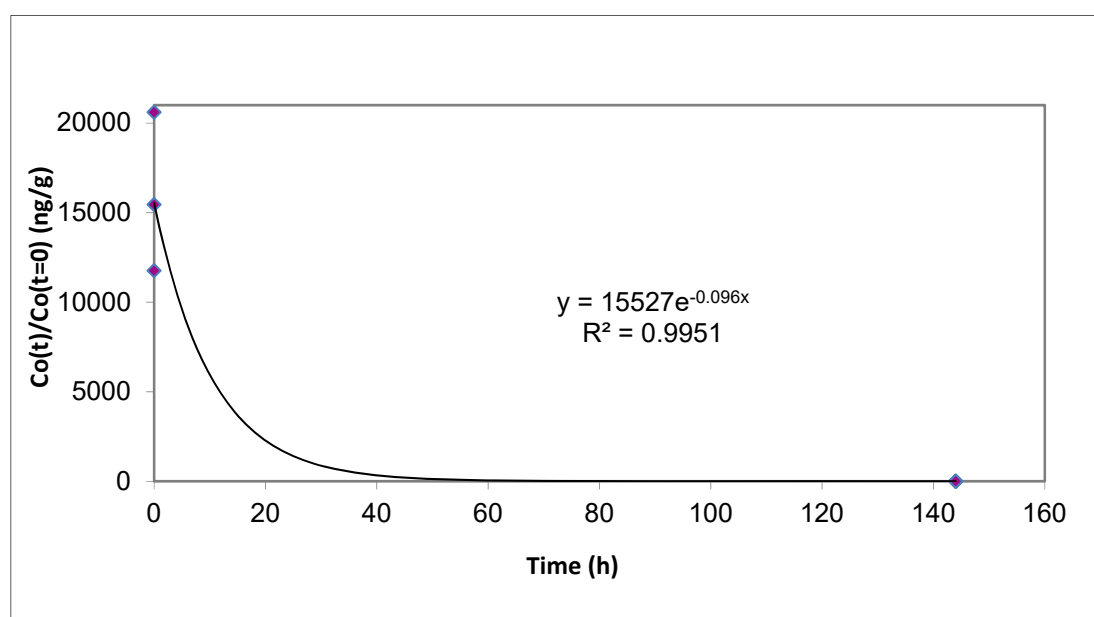


Figure 10. VEN rate constant of elimination calculated in mussels *M. galloprovincialis*.

Elimination percentage was obtained for each compound as described in section 5.3. For BEN, the percentage achieved was 99.81 ± 0.10 % and for VEN 98.23 ± 0.85 % after 6 days of depuration in clean water. It can be noticed from the k_2 curves (Figures 9 and 10), that only 48 hours were necessary for reaching approximately a 95% of depuration for both contaminants.

The half-life time of BEN and VEN in mussel was calculated with the formula described in the section 5.3. BEN showed an estimated half-life time equals to 1.55/d (37.2 h). This result is slightly higher than BEN half-life time previously described when sunlight impacts directly on the water (24 hours) (18). VEN showed a similar estimated half-life time equals to 1.60/d (38.4 h). This is slightly lower than the half life time reported for VEN in surface waters (57 hours).

7 CONCLUSIONS

During the 15 days that lasted the exposure period, concentrations of BEN and VEN in mussel were measured up to 1116.16 ng/g dw and 15937.83 ng/g dw, respectively for BEN and VEN, when they were exposed to average concentrations of 44.98 ng/mL of BEN and 19.42 ng/mL of VEN. A background level of BEN was found in mussels, in the control tanks and at the beginning of the experiment this indicates that organisms were exposed to the herbicide in their natural environment (Ebro Delta) as previously reported by other authors.

BEN and VEN showed low bioconcentration capacity in mussels during the *in vivo* experiment, exposed via water, under laboratory-controlled conditions. Both contaminants achieved their steady-state at 24 h, resulting in a BCF_{ss} of 5.74 L/Kg and 280.55 L/Kg for BEN and VEN respectively. Their Kinetic BCFs experimentally calculated were in the same order of magnitude, although smaller for BEN (0.74 L/Kg) and higher for VEN (439.22 L/Kg). Comparatively the BCFs determined for BEN is considerably lower than the one obtained for VEN in the same organism. This is mainly related to differences in their physical-chemical properties and the higher LogK_{ow} of VEN.

The rate constants of uptake (k_1) of BEN and VEN, were determined and their values were 1.65 (L/Kg d) and 1012.84 (L/Kg d) respectively. The rate constant of elimination (k_2) of both compounds were found to be similar 2.23/d and 2.30/d, respectively. The half-life time were also similar 1.55/d for BEN and 1.60/d for VEN. The results obtained for k_2 and half-life time support the high percentage of clearance reached for both contaminants after six days of depuration, resulting in 99.81 % for BEN and 98.23 % VEN.

The experiment has proved that both BEN and VEN are able to be bioconcentrated in wild mussels if an exposure in the natural environment is taking place (although they are considered as non-bioaccumulative compounds). However, their high depuration percentages assured the complete removal of the contaminants from mussel when the organism is placed in a contaminant-free environment. A short period of time, around 48h, would be enough to an almost complete depuration. Therefore, considering that mussel is depurated before commercialization, as soon as this time is achieved, a good quality of this seafood type would be assured. Regarding, the potential effects of their bioconcentration in the organism itself, more studies are required in order to evaluate their potential environmental risk.

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ACRONYMS

AMM	Artificial Marine Mesocosm
ACN	Acetonitrile
ASE	Pressurized liquid extraction
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
BCF _{ss}	Steady-state Bioconcentration Factor
BEN	Bentazone
CEC	Contaminants of Emerging Concern
dSPE	Solid phase dispersive extraction system
D _w	Dry weight
EC ₅₀	Half maximal effective concentration
EDC	Endocrine Disrupting Compounds
EPA	Environmental Protection Agency
GC/MS	Gas chromatography-mass spectrometry
HPLC	High-Pressure Liquid Chromatography
IDAEA-CSIC	Institute of Environmental Assessment and Water Research- Consejo Superior de Investigaciones Científicas.
IEO	Spanish Institute of Oceanography
IS	Internal Standard
MCPA	2-methyl-4-chlorophenoxyacetic acid
MDL	Method detection limit
MQL	Method quantification limit
MRL	Maximum residue limits
MS	Mass Spectrometry
NOEC	Non observed effect concentration
ODV	O-desmethylvenlafaxine
PhAC	Pharmaceutical Active Compound
RT	Retention time
QuEChERS	Quick, Easy, Cheap, Effective, Rugged & Safe)
UHPLC-HRMS	Ultra-High-Performance Liquid Chromatography-High Resolution Mass Spectrometry
VEN	Venlafaxine
WWTP	Waste Water Treatment Plant
W _w	Wet weight of the sample